REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
12-05-2015	Final Report	15-Feb-2014 - 14-Feb-2015

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Final Report: Instrumentation for Investigating the Regenerative Potential of Bone-Tissue-Engineered Scaffolds	W911NF-14-1-0075
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
	206022

6. AUTHORS	5d. PROJECT NUMBER
Elijah Nyairo	
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES	8. PERFORMING ORGANIZATION REPORT NUMBER
Alabama State University Biological Sciences Alabama State University Montgomery, AL 36101 -0271	

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)	10. SPONSOR/MONITOR'S ACRONYM(S) ARO
U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211	11. SPONSOR/MONITOR'S REPORT NUMBER(S) 64758-CH-REP.5

12. DISTRIBUTION AVAILIBILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

This research aims to develop polymeric nanofibers that can be used as tissue scaffolds. Nanoscale fiber scaffolds provide an optimal template for cells to seed, migrate and grow. The goal is for the cells to attach to the scaffolds, then replicate, differentiate and organize into normal healthy tissues as the scaffold degrades. In this study, non-woven Poly (?-caprolactone) (PCL)/Hydroxyapatite (HA) nanofibers and Poly (lactic-co-glycolic acid) (PLGA)/HA nanofibers with different wt % compositions were prepared by electrostatic co-spinning technology and characterized for bone tissue engineering applications. It was hypothesized that PCL/HA and PLGA/HA scaffolds

15. SUBJECT TERMS

Electrospinning, Nanofiber, Nanocomposite, Scaffolds, Electrophoretic Deposition

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Elijah Nyairo
UU	UU	UU	UU		19b. TELEPHONE NUMBER 334-229-6923

Standard Form 298 (Rev 8/98)
Prescribed by ANSI Std. Z39.18

Final Report: Instrumentation for Investigating the Regenerative Potential of Bone-Tissue-Engineered Scaffolds

ABSTRACT

This research aims to develop polymeric nanofibers that can be used as tissue scaffolds. Nanoscale fiber scaffolds provide an optimal template for cells to seed, migrate and grow. The goal is for the cells to attach to the scaffolds, then replicate, differentiate and organize into normal healthy tissues as the scaffold degrades. In this study, non-woven Poly (?-caprolactone) (PCL)/Hydroxyapatite (HA) nanofibers and Poly (lactic-co-glycolic acid) (PLGA)/HA nanofibers with different wt % compositions were prepared by electrostatic co-spinning technology and characterized for bone tissue engineering applications. It was hypothesized that PCL/HA and PLGA/HA scaffolds will mimic the nano-features of the natural extracellular matrix (ECM) and are expected to be effective as a matrix for cellular growth, proliferation and new tissue formation. To test if these scaffolds mimic the properties of natural ECM, we used TRAMP C1 and TRAMP C2 cell lines derived from transgenic adenocarcinoma of mouse prostate (TRAMP) mice. The scaffolds were analyzed by MTT assay at different time points to verify cell toxicity/proliferation. Characterization for morphology of the electrospun fibers were observed using scanning electron Microscopy (SEM) and SEM micrographs were analyzed using image analysis software. The fibers were characterized for thermal behavior using Differential Scanning Calorimetry (DSC), and for chemical structure using Fourier Transform Infrared Spectroscopy (FTIR).

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
08/29/2014	1.00	Amanee D Salaam, Manoj Mishra, Elijah Nyairo, Derrick Dean. Electrospun polyvinyl alcohol/nanodiamond composite scaffolds: morphological, structural, and biological analysis, Journal of Biomaterials and Tissue Engineering, (03 2014): 1. doi:
08/29/2014	2.00	Amanee D. Salaam, , Elijah Nyairo, , Vinoy Thomas, , Alyssa B. Terry,, Derrick R. Dean. PLGA Nanoparticles for the Sustained Release of Rifampicin, Nanogenomics and nanomedicine, (04 2014): 1. doi:
TOTAL:	**2**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

<u>Received</u>   <u>Paper</u>

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

<u>Received</u>   <u>Paper</u>

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

<u>Received</u>   <u>Paper</u>

TOTAL:

Number of Manuscripts:

Books

Received   Book

   TOTAL:

Received   Book Chapter

   TOTAL:

Patents Submitted

Patents Awarded

Awards

Participated in the Nicolet Continuum Microscope Operations Training Course. Thermo-Fisher Instruments: Unity Lab
Services, Madison, WI, 53711, Mar 23-27, 2015

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME   PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME   PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics
This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 2.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 2.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 2.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 1.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:...... 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME   PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Introduction

With the continual aging of the population in the United States, bone fractures and diseases such as osteoporosis, osteomalacia and osteitis deformans (Paget's disease of bone) present a need for the development and perfection of bone regeneration methods [1]. While numerous bone fractures can be treated by bone grafting, success rates vary, and the treatment is limited in several ways. Biodegradable bone tissue scaffolds may provide solutions that strike a balance between function and mechanical properties. Our interest in biodegradable polymers for bone tissue scaffolds includes controlling the polymer architecture through chemical modifications and preparing polymeric nanofiber scaffolds via electrospinning for tissue engineering.

The underlying principle of tissue engineering involves regenerating living tissue using cell-scaffold-based approaches [2 - 5]. Tissues can be potentially engineered by utilizing a combination of the patient's own cells combined with polymer scaffolds. One of the challenges in tissue engineering is the design of an ideal scaffold that can mimic the structure and biological functions of the natural extracellular matrix (ECM). Cells can attach and organize well around fibers with diameters smaller than those of the cell. Nanoscale fiber scaffolds provide an optimal template for cells to seed, migrate and grow. Polymeric nanofiber matrix is the most promising scaffolding materials for native ECM analogs [6, 7]. Electrospinning can produce polymeric fibers of nano-diameters to mimic the nanoscale features of native ECM. While a significant number of studies have investigated biodegradable polymers for bone tissue scaffolds, one problem which has not been solved is the mismatch between the mechanical properties of the bone and the scaffold materials. Thus, our objective is to develop biodegradable tissue scaffolds for bone that mimics the size scale and chemistry of the ECM with nanometer dimensions, an interconnected pore structure, and enhanced mechanical properties. We will investigate interesting combinations of constituent materials and modifications of the electrospinning process to develop polymeric scaffolds with varying degrees of hierarchical complexity and functionality, and evaluate their propensity for cell growth.

In this study, scaffolds using PLGA/HA and PCL/HA nanofibers with different wt % compositions were fabricated via electrostatic co-spinning technology and characterized for bone tissue engineering applications. The inherent nonwoven nature of the electrospun nanofibers result in interconnected pores sufficient for cell attachment [8, 9]. The process of electrospinning involves an applied electric field to form fibers from a polymer solution. The size, properties, and quality of the fibers will differ depending on the concentration of the solution and process parameters of electrospinning (applied voltage, needle size, volume feed rate, distance of the collector plate from the needle tip, as well as the solution characteristics i.e., viscosity, conductivity). The formation of nanofibers by electrospinning is induced via the uniaxial stretching of a viscoelastic solution.

Polycaprolactone (PCL), biodegradable polyester, has received US FDA approval for several clinical applications for humans and is also a commonly used scaffold for tissue engineering due to its high biocompatibility, mechanical properties and nontoxicity. However, the drawbacks of PCL, such as its strong hydrophobicity, slow degradation kinetics, and lack of bioactive functions, have greatly limited its application as scaffolds in tissue engineering. Therefore, blending the bioactive functions of hydroxyapatite with the good mechanical properties of PCL to generate a new biohybrid material should show an improvement in biological, mechanical, and degradation properties compared with the individual components.

Hydroxyapatite (HA) is osteoconductive, nontoxic, noninflammatory, and promotes cell proliferation. In addition, it has been reported that hydroxyapatite can increase the compressive strength of PCL [10, 11]. Furthermore, as one of the natural components in bone, hydroxyapatite imitates the natural bone extracellular matrix, increasing surface area, wettability, and roughness, to optimize bone cell-specific protein interaction with cells.

Methods
Materials:

PLGA copolymer with lactide to glycolide ratio of 85/15 and inherent viscocity 0.63 dL/g was obtained from Lactel Absorbable Polymers (AL, USA). PCL polymer with an inherent viscosity 1.15 dL/g aws obtained from Durect Corporation, (Cupertino, CA). Hydroxyapatite (HA) powder with an average diameter of 100 nm-150 nm particle size and 15 m2/g surface area were purchased from Nanocerox Inc. (Ann Arbor, MI). Methanol (CH3OH), Chloroform (CH3Cl), Dichloromethane (DCM) and Dimethylformamide (DMF) were obtained from Fisher Scientific (Fair Lawn, NJ). All solvents were used as received without further purification.

Solution Preparation:

Different compositions of PLGA/HA 1%, PLGA/HA 5% and neat PLGA were prepared in CH2Cl2. PCL/HA solutions were prepared where HA particles (1.0, 5.0 and 10.0 wt %) were mixed with PCL first under mechanical stirring in DMF to ensure a good dispersion of HA particles within the resulting. DCM was added and the mixture stirred and sonicated until the PCL powder dissolved completely to form a precursor dispersion for electrospinning nanofibers.

The polymers were dissolved using a magnetic stirrer to achieve a ~15.0-wt% solution and electrospun at a high voltage (GAMMA High Voltage Research, Ormondo Beach, FL) of 15 kV to create nanofibers. A feeding rate of 1.5 mL/hr (0.025 mL/min) was set in an F100 Syringe Pump (Chemyx Inc, Houston TX) and a grounded aluminum collector plate was placed about 10 cm from the tip of the needle.

Electrospinning Process:

Electrospinning is a process that is used to produce nanofibers from solution. To generate fibers, a polymer solution is forced through a capillary, forming a drop of polymer solution at the tip. Charges (~17 kV) from a high voltage electrical field are induced on the liquid droplet held by its surface tension. This is done by connecting one electrode to a syringe containing the polymer solution and another to a collector plate. As the intensity of the electric field increases, surpassing a threshold value, the droplet elongates, the electrostatic force between the needle and the collector plate overcomes the surface tension, and a streaming jet of the solution is ejected from the tip of the needle. The solvent evaporates leaving behind a charged thin polymer fiber. Fibers are deposited in a random orientation when the collector is a flat metal plate. Fibers can be deposited in a rotating target when the collector is modified to incorporate a rotating metal plate on which the polymer is wound. The resulting fibers can have diameters on the order of tens to hundreds of nanometers. In electrospinning, the key to optimal properties is significant elongation of the polymer. As the electrospinning jet moves away from the nozzle, it is stretched by whipping and bending instabilities. This results in an extremely large draw ratio, which serves to elongate and align the polymer chains.

Characterization

Fourier Transform-Infrared (FT-IR) spectra were recorded in attenuated total reflection (ATR) mode using a Thermo Nicolet iS50 FT-IR Spectrophotometer (DOD W911NF-14-1-0075). The spectra were obtained with 32 scans per sample ranging from 4000 to 400 cm-1. Spectra showed an ester carbonyl stretch from the PLGA and PCL (C=O) at 1747 cm-1 in all the scaffolds with no significant shift due to HA interaction. Other major peaks observed were the C-O-C ether group at 1083 cm-1, C-O stretch at 1128 cm-1, O-H deformation at 1264 cm-1, methyl group C-H stretching at 1452 cm-1 and other methylene, methyl groups at 2800–3300 cm-1. For the nanocomposite scaffolds a PO_4^{3-} stretch (1030 cm-1) and bending (570 cm-1), typical of HA were observed with intensities varying proportionally to the HA concentration.

Thermal analyses of the scaffolds were evaluated using a TA Instrument DSC Q2000 differential scanning calorimetry (DOD W911NF-06-1-0497). DSC was used to study the thermal behavior of the nanofibers. 5.0-10.0 mg cured samples were heated from room temperature to 250°C with a heating rate of 10°C/min. The sample chamber will be purged with nitrogen gas at a flow rate of 50mL/h.

The morphology of the fibers is currently under evaluation using scanning electron microscopy (SEM). The fiber diameter distributions in the scaffolds will be obtained by analyzing the SEM micrographs using image-analysis software. It is expected that the diameter distributions of PLGA/HA nanofibers will be well within the range of the natural ECM (50–500 nm) [8, 12-15].

Cytotoxic properties of nanofiber scaffolds evaluated in vitro cell culture system. TRAMP C1 cells were treated with different percent of PLGA/HA for 24 hours and cell toxicity was analyzed by MTT assay. TRAMP C2 cells were seeded on neat PCL and PCL with different percentage of HA for 24 hours at 37 0C with 5% CO2. Cell viability was measured using the methyl thiazoletetrazolium (MTT) assay. Figure 4 demonstrates that the viability of TRAMP cells was not compromised even at higher percentage of PLGA/HA and PCL/HA. Thus, this data suggests that PLGA/HA and PCL/HA can be used a therapeutic carrier without compromising the viability of cells.

Technology Transfer

Abstract

This research aims to develop polymeric nanofibers that can be used as tissue scaffolds. Nanoscale fiber scaffolds provide an optimal template for cells to seed, migrate and grow. The goal is for the cells to attach to the scaffolds, then replicate, differentiate and organize into normal healthy tissues as the scaffold degrades. In this study, non-woven Poly (e-caprolactone)/Hydroxyapatite (PCL/HA) nanofibers and Poly (lactic-co-glycolic acid) (PLGA)/HA nanofibers with different wt % compositions were prepared by electrostatic co-spinning technology and characterized for bone tissue engineering applications. It was hypothesized that PCL/HA and PLGA/HA scaffolds will mimic the nano-features of the natural extracellular matrix (ECM) and are expected to be effective as a matrix for cellular growth, proliferation and new tissue formation. To test if these scaffolds mimic the properties of natural ECM, we used TRAMP C1 and TRAMP C2 cell lines derived from transgenic adenocarcinoma of mouse prostate (TRAMP) mice. The scaffolds were analyzed by MTT assay at different time points to verify cell toxicity/proliferation. Characterization for morphology of the electrospun fibers were observed using scanning electron Microscopy (SEM) and micrographs were analyzed using image analysis software. The fibers were characterized for thermal behavior using Differential Scanning Calorimetry (DSC), and for chemical structure using Fourier Transform Infrared Spectroscopy (FTIR).

Introduction

With the continual aging of the population in the United States, bone fractures and diseases such as osteoporosis, osteomalacia and osteitis deformans (Paget's disease of bone) present a need for the development and perfection of bone regeneration methods [1]. While numerous bone fractures can be treated by bone grafting, success rates vary, and the treatment is limited in several ways. Biodegradable bone tissue scaffolds may provide solutions that strike a balance between function and mechanical properties. Our interest in biodegradable polymers for bone tissue scaffolds includes controlling the polymer architecture through chemical modifications and preparing polymeric nanofiber scaffolds via electrospinning for tissue engineering.

The underlying principle of tissue engineering involves regenerating living tissue using cell-scaffold-based approaches [2 - 5]. Tissues can be potentially engineered by utilizing a combination of the patient's own cells combined with polymer scaffolds. One of the challenges in tissue engineering is the design of an ideal scaffold that can mimic the structure and biological functions of the natural extracellular matrix (ECM). Cells can attach and organize well around fibers with diameters smaller than those of the cell. Nanoscale fiber scaffolds provide an optimal template for cells to seed, migrate and grow. Polymeric nanofiber matrix is the most promising scaffolding materials for native ECM analogs [6, 7]. Electrospinning can produce polymeric fibers of nano-diameters to mimic the nanoscale features of native ECM. While a significant number of studies have investigated biodegradable polymers for bone tissue scaffolds, one problem which has not been solved is the mismatch between the mechanical properties of the bone and the scaffold materials. Thus, our objective is to develop biodegradable tissue scaffolds for bone that mimics the size scale and chemistry of the ECM with nanometer dimensions, an interconnected pore structure, and enhanced mechanical properties. We will investigate interesting combinations of constituent materials and modifications of the electrospinning process to develop

polymeric scaffolds with varying degrees of hierarchical complexity and functionality, and evaluate their propensity for cell growth.

In this study, scaffolds using PLGA/HA and PCL/HA nanofibers with different wt % compositions were fabricated via electrostatic co-spinning technology and characterized for bone tissue engineering applications. The inherent nonwoven nature of the electrospun nanofibers result in interconnected pores sufficient for cell attachment [8, 9]. The process of electrospinning involves an applied electric field to form fibers from a polymer solution. The size, properties, and quality of the fibers will differ depending on the concentration of the solution and process parameters of electrospinning (applied voltage, needle size, volume feed rate, distance of the collector plate from the needle tip, as well as the solution characteristics i.e., viscosity, conductivity). The formation of nanofibers by electrospinning is induced via the uniaxial stretching of a viscoelastic solution.

Polycaprolactone (PCL), biodegradable polyester, has received US FDA approval for several clinical applications for humans and is also a commonly used scaffold for tissue engineering due to its high biocompatibility, mechanical properties and nontoxicity. However, the drawbacks of PCL, such as its strong hydrophobicity, slow degradation kinetics, and lack of bioactive functions, have greatly limited its application as scaffolds in tissue engineering. Therefore, blending the bioactive functions of hydroxyapatite with the good mechanical properties of PCL to generate a new biohybrid material should show an improvement in biological, mechanical, and degradation properties compared with the individual components.

Hydroxyapatite (HA) is osteoconductive, nontoxic, noninflammatory, and promotes cell proliferation. In addition, it has been reported that hydroxyapatite can increase the compressive strength of PCL [10, 11]. Furthermore, as one of the natural components in bone, hydroxyapatite imitates the natural bone extracellular matrix, increasing surface area, wettability, and roughness, to optimize bone cell-specific protein interaction with cells.

Methods

Materials:

PLGA copolymer with lactide to glycolide ratio of 85/15 and inherent viscocity 0.63 dL/g was obtained from Lactel Absorbable Polymers (AL, USA). PCL polymer with an inherent viscosity 1.15 dL/g aws obtained from Durect Corporation, (Cupertino, CA). Hydroxyapatite (HA) powder with an average diameter of 100 nm-150 nm particle size and 15 m2/g surface area were purchased from Nanocerox Inc. (Ann Arbor, MI). Methanol (CH3OH), Chloroform (CH3Cl), Dichloromethane (DCM) and Dimethylformamide (DMF) were obtained from Fisher Scientific (Fair Lawn, NJ). All solvents were used as received without further purification.

Solution Preparation:

Different compositions of PLGA/HA 1%, PLGA/HA 5% and neat PLGA were prepared in CH_2Cl_2. PCL/HA solutions were prepared where HA particles (1.0, 5.0 and 10.0 wt %) were mixed with PCL first under mechanical stirring in DMF to ensure a good dispersion of HA particles within the resulting. DCM was added and the mixture stirred and sonicated until the PCL powder dissolved completely to form a precursor dispersion for electrospinning nanofibers.

The polymers were dissolved using a magnetic stirrer to achieve a ~15.0-wt% solution and electrospun at a high voltage (GAMMA High Voltage Research, Ormondo Beach, FL) of 15 kV to create nanofibers. A feeding rate of 1.5 mL/hr (0.025 mL/min) was set in an F100 Syringe Pump (Chemyx Inc, Houston TX) and a grounded aluminum collector plate was placed about 10 cm from the tip of the needle.

Electrospinning Process:

Electrospinning is a process that is used to produce nanofibers from solution. To generate fibers, a polymer solution is forced through a capillary, forming a drop of polymer solution at the tip. Charges (~17 kV) from a high voltage electrical field are induced on the liquid droplet held by its surface tension. This is done by connecting one electrode to a syringe containing the polymer solution and another to a collector plate. As the intensity of the electric field increases, surpassing a threshold value, the droplet elongates, the electrostatic force between the needle and the collector plate overcomes the surface tension, and a streaming jet of the solution is ejected from the tip of the needle. The solvent evaporates leaving behind a charged thin polymer fiber. Fibers are deposited in a random orientation when the collector is a flat metal plate. Fibers can be deposited in a rotating target when the collector is modified to incorporate a rotating metal plate on which the polymer is wound. The resulting fibers can have diameters on the order of tens to hundreds of nanometers. In electrospinning, the key to optimal properties is significant elongation of the polymer. As the electrospinning jet moves away from the nozzle, it is stretched by whipping and bending instabilities. This results in an extremely large draw ratio, which serves to elongate and align the polymer chains.
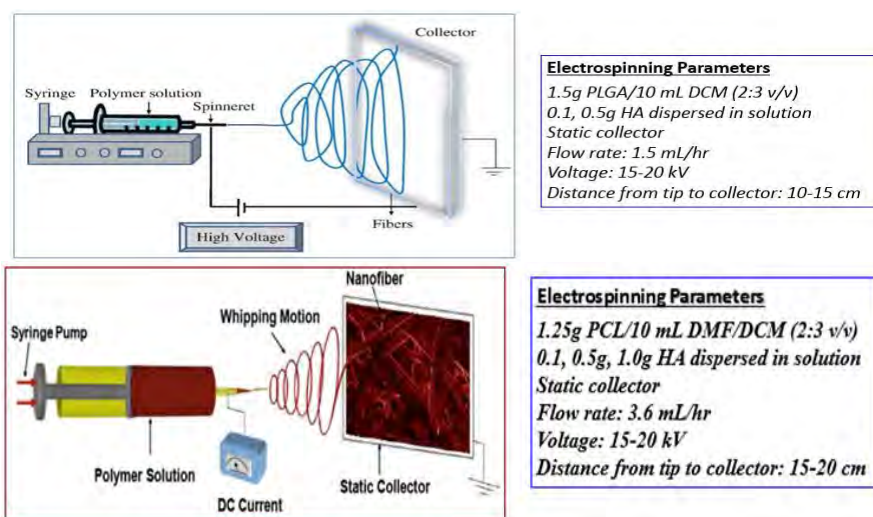

Fig 1: Electrospinning Set-up for collecting randomly aligned Nanofibers

Results

Characterization

Fourier Transform-Infrared (FT-IR) spectra were recorded in attenuated total reflection (ATR) mode using a Thermo Nicolet iS50 FT-IR Spectrophotometer (DOD W911NF-14-1-0075). The spectra were obtained with 32 scans per sample ranging from 4000 to 400 cm-1. Spectra showed an ester carbonyl stretch from the PLGA and PCL (C=O) at 1747 cm^{-1} in all the scaffolds with no significant shift due to HA interaction. Other major peaks observed were the C-O-C ether group at 1083 cm-1, C-O stretch at 1128 cm-1,O-H deformation at 1264 cm-1, methyl group C-H stretching at 1452 cm-1 and other methylene, methyl groups at 2800–3300 cm-1. For the nanocomposite scaffolds a PO4 3- stretch (1030 cm-1) and bending (570 cm-1), typical of HA were observed with intensities varying proportionally to the HA concentration.
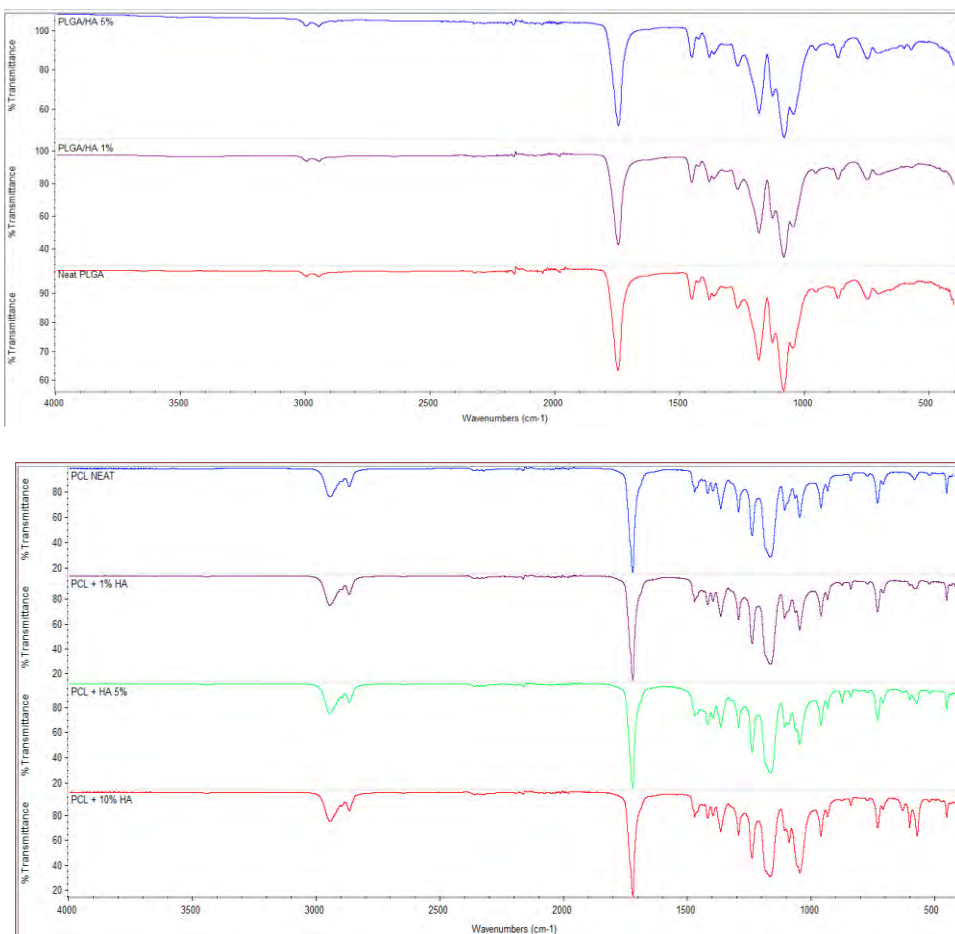

Fig 2: FTIR-ATR spectra of Polymer Nanocomposites

Thermal analyses of the scaffolds were evaluated using a TA Instrument DSC Q2000 differential scanning calorimetry (DOD W911NF-06-1-0497). DSC was used to study the thermal behavior of the nanofibers. 5.0-10.0 mg cured samples were heated from room temperature to 250°C with a heating rate of 10°C/min. The sample chamber will be purged with nitrogen gas at a flow rate of 50mL/h.
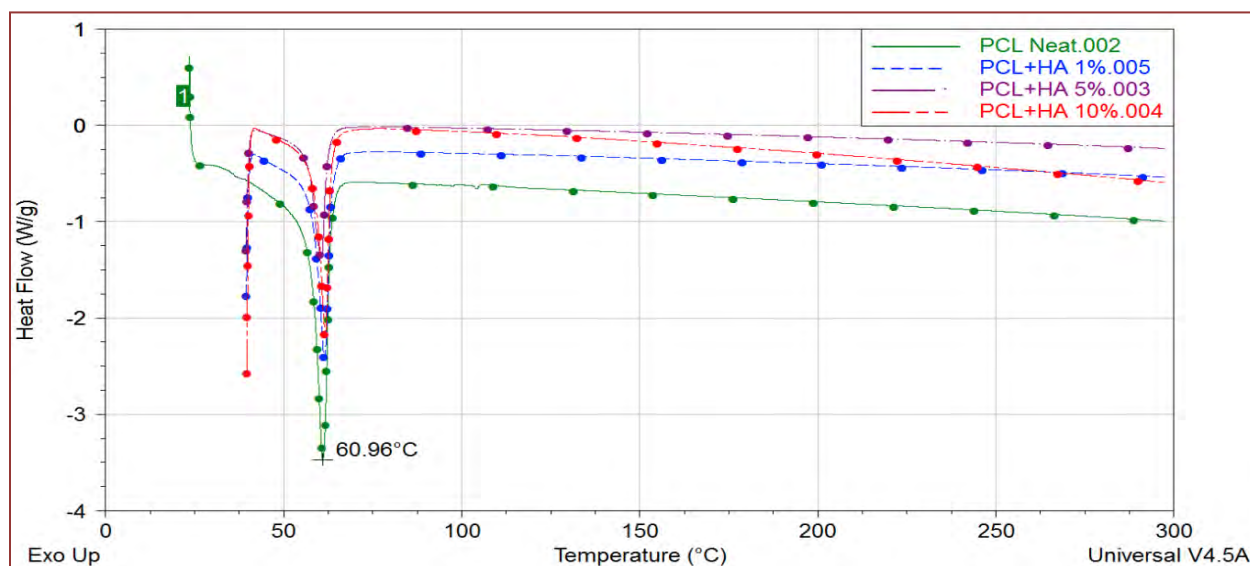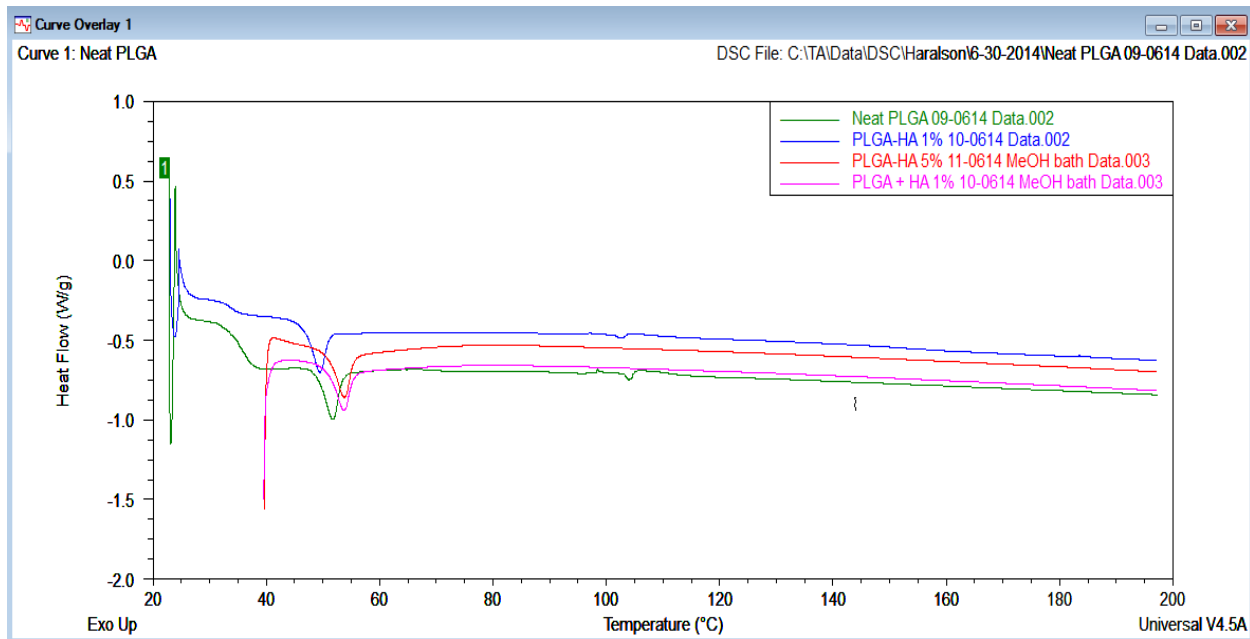

Fig 3: DSC Thermal Behavior Data for PLGA and PCL Scaffolds

The morphology of the fibers is currently under evaluation using scanning electron microscopy (SEM). The fiber diameter distributions in the scaffolds will be obtained by analyzing the SEM micrographs using image-analysis software. It is expected that the diameter distributions of PLGA/HA nanofibers will be well within the range of the natural ECM (50–500 nm) [8, 12-15].

Cytotoxic properties of nanofiber scaffolds evaluated *in vitro* cell culture system. TRAMP C1 cells were treated with different percent of PLGA/HA for 24 hours and cell toxicity was analyzed by MTT assay. TRAMP C2 cells were seeded on neat PCL and PCL with different percentage of HA for 24 hours at 37 ^{0}C with 5% CO2. Cell viability was measured using the methyl thiazoletetrazolium (MTT) assay.
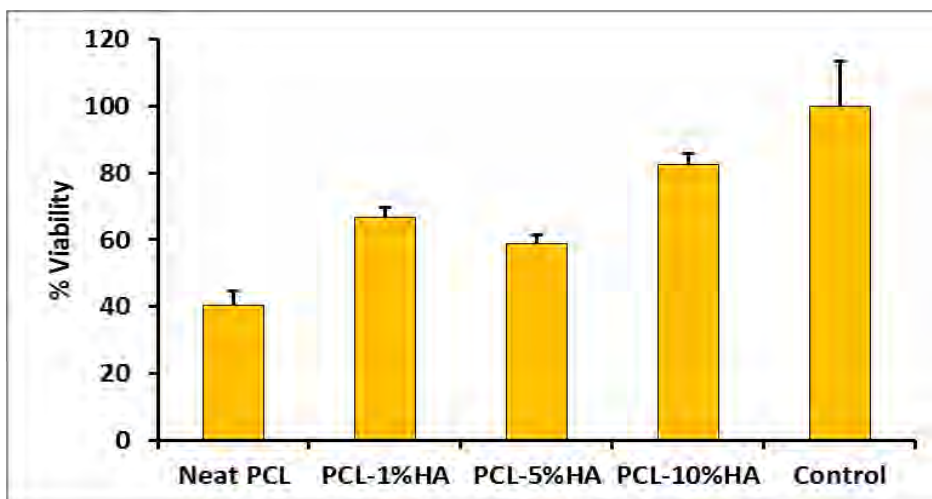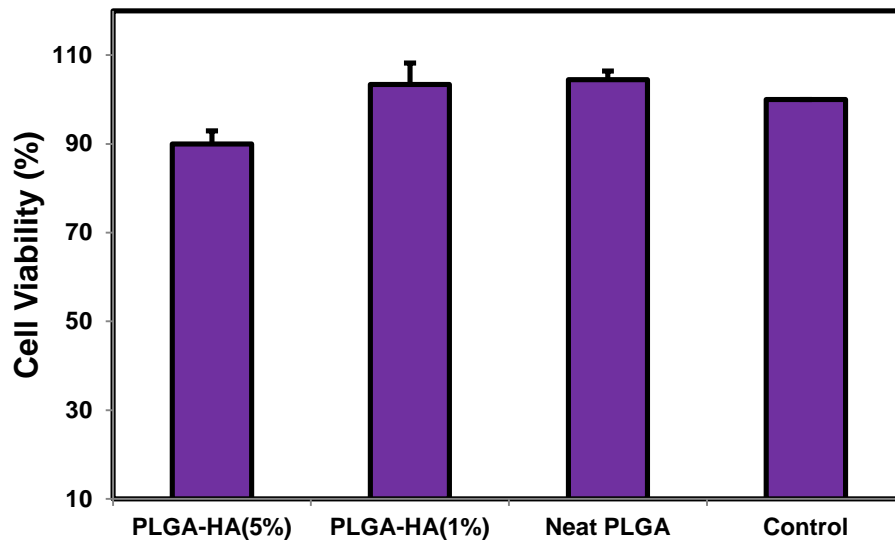

Fig 4: Effect of different concentrations of PLGA/HA and PCL/HA scaffolds on cell viability.

Figure 4 demonstrates that the viability of TRAMP cells was not compromised even at higher percentage of PLGA/HA and PCL/HA. Thus, this data suggests that PLGA/HA and PCL/HA can be used a therapeutic carrier without compromising the viability of cells.

References:

1. Lanza, R., Langer, R., and Vacanti, J., <u>Principles of Tissue Engineering</u>, second edition, Academic Press, 2000.
2. Hansbrough JF, Morgan J, Greenleaf G. Advances in wound coverage using cultured cell technology. *Wounds*, 1993; **5**: 174-194.
3. Hellman KB. Bioartificial organs as outcomes of tissue engineering. Science and regulatory issues. Ann N Y Acad Sci 1997; **831**: 1-14.
4. Heimbach D, Luterman A, Burke JF. Artificial dermis for major burns. A multi-center randomized   clinical trial. *Ann Surg* 1988; **208**: 313-320.
5. Yannas IV. Tissue regeneration by use of collagen-glycosaminoglycan copolymers. *Clin. Mater*. 1992: **9**: 179-187.
6. Z. Ma *et al*. *Tissue Engg*. 11,   101 (2005).
7. H. Yoshimoto *et al. Biomaterials* 24, 2077 (2003).
8. Li W-J, Laurencin CT, Caterson EJ, Tuan RS, Ko FK. Electrospun nanofibrous structure: A novel scaffold for tissue engineering. J Biomed Mater Res 2002;60:613-21.
9. Huang Z-M, Zhang, Y. Z. Kotaki M., Ramakrishna, S. A review on polymer nano-fibers by electrospinning and their applications in nanocomposites. Compos Sci Technol 2003;63:2223-53.
10. Barbara et al.*, Advanced Functional Materials.* 17, 13 (2007) pp. 2180 – 2188
11. Thomas et al., *Biomacromolecules.* 8, 2(2007) 631-637
12. Mikos AG, Temenoff JS. Formation of highly porous biodegradable scaffolds for tissue engineering. Electron J Biotechnol 2000;3:114-19.
13. Laurencin CT, Ambrosio AMA, Borden MD, Cooper JA. Tissue Engineering: Or-thopedic Applications. Annu Rev Biomed Eng 1999;1:19.
14. Murugan R, Ramakrishna S. Nano-Featured Scaffolds for Tissue Engineering: A Review of Spinning Methodologies. Tissue Eng 2006;12:435-47.
15. Quynh P. Pham, Upma Sharma, and Dr. Antonios G. Mikos. Tissue Engineering. May 2006, 12(5): 1197-1211. doi:10.1089/ten.2006.12.1197: 1202-08